# PET imaging of brain sex steroid hormone receptors and the role of estrogen in depression

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## Chapter 5

# The Effect of Estrogen Replacement on Neural Responses of Rats to Stress in the Forced Swim Test

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**Abstract**

Reduced levels of circulating estrogens have been associated with declined cognition, anxiety and depression. In post-menopausal women, hormone replacement therapy with estrogens was found to have an antidepressant effect and to enhance the effect of other antidepressant drugs. However, there appears to be a critical window for hormone replacement therapy, which depends on age and the stage of menopause. In this study, the effects of estrogen replacement on depressive-like behavior and brain metabolism were studied in ovariectomized female rats as a model for post-menopausal depression. Estradiol replacement was started immediately or 7-days after ovariectomy and compared with sub-acute treatment with the antidepressant drugs fluoxetine and escitalopram. The antidepressant effect of treatment was evaluated by the forced swim test (FST) and brain glucose metabolism was measured in rest and during stress in the FST by positron emission tomography (PET) with 2-deoxy-2-[$^{18}$F]fluoro-D-glucose ([$^{18}$F]FDG). Estradiol replacement had a beneficial effect on depressive-like behavior when started immediately after ovariectomy, but not after a 7-day delay. Immediate estradiol replacement reduced whole brain [$^{18}$F]FDG uptake in the resting state, but not during stress. No regional differences in brain metabolism between groups were found in the resting state. However, during stress in the FST, immediate, but not delayed, estradiol replacement resulted in higher relative glucose metabolism in periaqueductal grey, superior colliculus and cerebellum and lower metabolism in caudate putamen and corpus callosum than placebo treatment. Thus, only estradiol replacement immediately after ovariectomy could produce a significant antidepressant effect, which was accompanied by altered brain glucose metabolism.

## Introduction

Estrogens are known for their role in reproductive function in females, but they are also associated with changes in cognitive function, anxiety and depression. Post-menopausal women can experience decreased cognitive function and depression as a consequence of a decline in circulating estrogens[1]. Therefore, estrogen replacement may be used as treatment for depression in post-menopausal women and as a means to improve cognitive function[2]. Estrogens not only possess antidepressant properties, but can also enhance the effect of antidepressant drugs, such as selective serotonin reuptake inhibitors (SRRIs)[3,4]. However, there appears to be a critical window for effective estrogen replacement therapy, which depends on the age of the patient and the stage of menopause[3,5,6]. The effects of declining estrogen levels have also been studied in animal models. Depletion of circulating estrogens by ovariectomy in rats is accompanied by the occurrence of depressive-like symptoms within one week after surgery. These effects of ovariectomy could be reduced by estrogen replacement, provided is started timely. When estrogen replacement was started 12 weeks after ovariectomy, the efficacy of the treatment was completely lost[7].

Several studies have demonstrated antidepressant properties of estrogens and the dependence of estrogen replacement on the time of initiation, but the effect of estrogens on brain metabolism is not well studied. This is of interest as depression is associated with decreased brain glucose metabolism[8–10]. Treatment of depression with for example SSRIs, which increase the synaptic concentrations of serotonin, was found to normalize brain glucose metabolism, especially in brain regions that play a role in serotonergic neurotransmission[11,12]. Likewise, estrogen replacement was found to restore normal brain glucose metabolism in post-menopausal women[13,14]. Measurement of brain glucose metabolism may thus be suitable biomarker to assess the efficacy of estrogen replacement.

In this study, we investigated how the timing of estrogen replacement affected depressive-like behavior measured in the forced swim test[15] (FST) and brain glucose metabolism in ovariectomized rats. Brain metabolism was studied with small animal positron emission tomography (PET), using the glucose analog 2-deoxy-2-[$^{18}$F]fluoro-D-glucose ([$^{18}$F]FDG) and was measured both in resting state and during stress (i.e. during the FST). It was hypothesized that a reduction in circulating estrogens has a detrimental effect on the ability of the animal to cope with stress and that this vulnerability could be reversed by estradiol replacement. The effect of estradiol replacement on depressive-like behavior and brain glucose metabolism was compared with the effect of the SSRIs fluoxetine and escitalopram.

## Materials and methods

### Animals

Female outbred Wistar rats (n=40, 9-12 weeks old, 200-250 g) were obtained from Harlan (Horst, The Netherlands). The rats were housed in pairs in Macrolon cages on a layer of wood shavings in a room with constant temperature (21±2$^{o}$C), and a fixed 12 h light-dark regime. Standard laboratory chow and water were available ad

libitum. After arrival, the rats were allowed to acclimatize for at least 7 days. During the acclimatization period and throughout the study, all rats were handled daily. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Groningen, The Netherlands (study protocol: DEC 5842B).

## Study design

All rats were ovariectomized and divided into 5 groups (n=8 per group): 1) placebo (OVX); 2) 7-day estradiol treatment starting on day 7 post-ovariectomy (OVX-7d-E2), 3) 14-day estradiol treatment starting on day 0 post-ovariectomy (OVX-14d-E2), 4) 24 h treatment with fluoxetine (OVX-FLX); and 5) 24 h treatment with escitalopram (OVX-ESC). The experimental procedures started 14 days post-ovariectomy with a resting state [$^{18}$F]FDG PET scan of the brain (Figure 1). On day 15 post-ovariectomy, a 15 min pretest-FST session was performed. After 24 h (day 16), the test-FST session of 30 min was performed, which was followed by a [$^{18}$F]FDG PET scan. Before the test-FST session, rats were injected intra-peritoneally with [$^{18}$F]FDG and immediately subjected to the FST so that the brain activity of the rats during the FST (stress condition) could be measured. Immediately after the PET scan during stress, blood was collected by cardiac puncture for plasma estradiol measurements and rats were terminated.

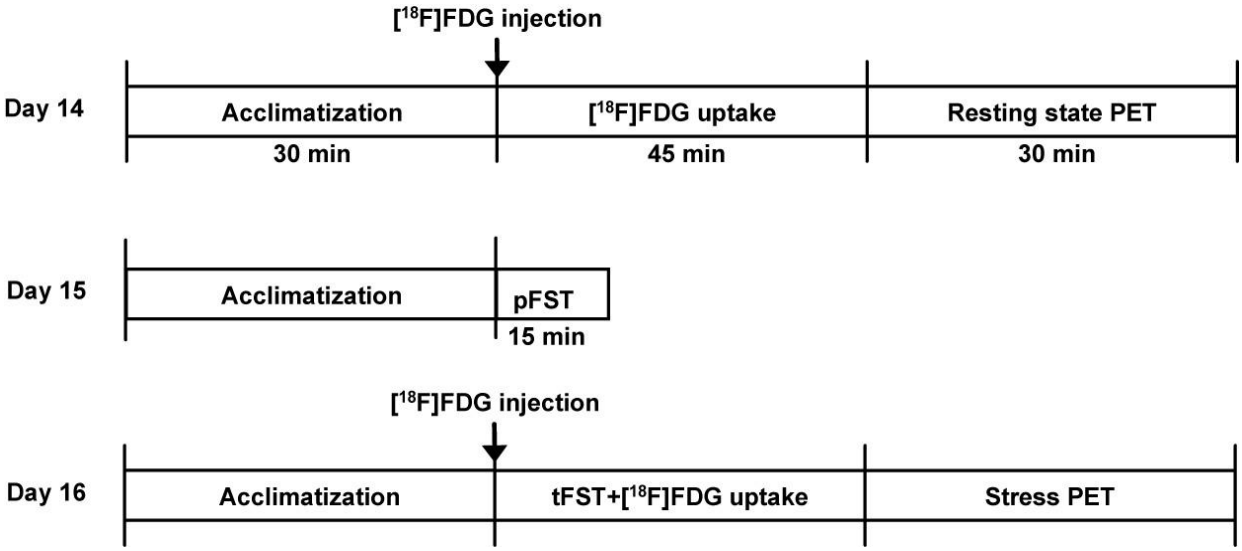


**Figure 1:** Study design, showing the timelines of [$^{18}$F]FDG PET and the forced swim test (FST). pFST = pre-test FST; tFST = test-FST

## Surgery

Bilateral ovariectomy was performed as previously described[16]. After ovariectomy, a placebo or estradiol pellet was implanted subcutaneously in the neck region. Body temperature was measured from day 12 until 16 post-ovariectomy to study the effect of treatment, anesthesia and the FST. To measure body temperature, an iButton with a sensitivity of 0.0625 $^{o}$C (DS1922L#F50, Maxim Integrated, San Jose, CA, USA)[17] was implanted into the abdominal cavity. iButtons were sealed in paraffin wax, sterilized by 24 h incubation in 70% ethanol prior to implantation and programmed

to measure body temperature of the rats every 90 s. Immediately after surgery, rats received a subcutaneous injection of finadyne (Flunixin 2.5 mg/kg, Schering-Plough N.V/S.A., Belgium) for pain relief, which was repeated 24 h after surgery.

**Treatment**

On the day of ovariectomy (day 0), the rats were subcutaneously implanted with either a placebo or an estradiol-releasing pellet (0.36 mg of 17ß-estradiol per pellet, 14 day release, Innovative Research of America, USA) in the neck. On day 7 post-ovariectomy, the pellet was replaced under anesthesia by either a placebo or an estradiol-releasing pellet. Rats in the OVX, OVX-FLX and OVX-ESC groups were implanted with a placebo pellet both on day 0 and on day 7, rats in the OVX-7d-E2 group were implanted with a placebo pellet on day 0 followed by an estradiol-releasing pellet on day 7 and rats in the OVX-14d-E2 group were implanted with an estradiol-releasing pellet both on day 0 and on day 7. Rats in the OVX-FLX and OVX-ESC group received an intra-peritoneal injection of 10 mg/kg fluoxetine or escitalopram in water for injection at 24, 19 and 1 h prior to the test-FST session on day 16 [12,18]. All other groups received vehicle injections on the same time-points.

**Estradiol measurements**

The levels of circulating estradiol were measured in rats from the OVX, OVX-7d-E2 and OVX-14d-E2 groups. It was assumed that the ovariectomized rats that were treated with fluoxetine and escitalopram had similar circulating estradiol levels as the OVX group, so these groups were not measured. Estradiol measurement was performed by an automatic immunoassay system (AutoDELFIA, Perkin Elmer).

**The forced swim test and positron emission tomography acquisition**

On day 14 post-ovariectomy, all rats were subjected to a 30 min static [$^{18}$F]FDG PET scan (resting state). The rats were placed in a pre-warmed cage (30 °C) for 30 min for environmental adaptation and minimization of [$^{18}$F]FDG uptake by brown fat. After this period, the rats were injected intra-peritoneally with [$^{18}$F]FDG (17.0±3.4 MBq) and placed back in the pre-warmed cage. At 30 min post-injection, rats were anesthetized with isoflurane mixed with medical air (5% induction and 2.5% maintenance) and positioned into the small animal PET camera (Focus 220, Siemens Medical Solutions, USA, Inc) in a trans-axial position with their heads in the field of view. At 45 min after [$^{18}$F]FDG injection, a static emission scan of 30 min was started. During the scan, the body temperature of the rats was maintained using heating pads. After the emission scan, a 515 s transmission scan with a $^{57}$Co point source was obtained for correction of attenuation and scatter by tissue.

On day 15 post-ovariectomy, all rats were subjected to the pretest-FST. The rats were placed in pre-warmed cages for 30 min and subsequently forced to swim for 15 min in a cylindrical tank (40 cm in height, 20 cm in diameter) filled with water maintained at 30 $^{o}$C. The water in the tank was 30 cm deep, allowing the rats to swim or float without touching the bottom of the tank with their hind limbs. After

completion of the pretest session, the animals were dried with paper towels and placed back into their home-cages. After every swim session, the water tank was cleaned and refilled with fresh water for the next rat.

On day 16 post-ovariectomy, all rats were subjected to the test-FST. The rats were first placed in a pre-warmed cage for 30 min. Immediately after intraperitoneal injection of [$^{18}$F]FDG, rats were forced to swim for 30 min in the cylindrical tank filled with water maintained at 30 $^{o}$C. After the 30 min FST, rats were dried with paper towels, anesthetized with isoflurane and subjected to a PET scan (stress), as described above for the resting state PET scan.

Both the pretest- and the test-FST sessions were recorded on video. Behavioral analysis was performed using Ethovision XT 8 (Noldus Information Technology, Wageningen, The Netherlands). The duration of passive behavior (floating) and active behavior (swimming and climbing) were measured. An increased amount of time spent on floating is regarded an indication of depressive-like behavior, i.e. a behavioral correlate of negative mood.

**Positron emission tomography image reconstruction and analysis**

List mode emission data was iteratively reconstructed into a single frame image of 30 min (OSEM2D, 4 iterations, and 16 subsets). PET data were normalized and corrected for attenuation, scatter, random coincidences and radioactive decay.

Whole brain region-of-interest (ROI) based analysis was performed to determine global functional changes as a consequence of treatment (resting state) and stress (during FST), using Inveon Research Workplace 4.0 (Siemens Medical Solutions, USA, Inc). For this purpose, the static PET images were co-registered with a T2-weigthed MRI rat brain template[19]. A region of interest of the whole brain was drawn on the MRI template and transferred to co-registered PET images. The [$^{18}$F]FDG uptake in the whole brain was measured as Bq/cm$^3$ and converted into the standardized uptake value (SUV), which was defined as: [tissue activity concentration (Bq/cm$^3$)]/[injected dose (Bq)/body weight (g)]. It was assumed that 1 cm$^3$ of brain tissue equals 1 g.

To determine regional differences in brain metabolism, voxel-based analysis was performed using SPM8 software (SPM; Wellcome Department of Cognitive Neurology, University College London, UK), in combination with an in-house toolbox that allows the visualization of the results over a rat 'glass brain' (maximum intensity projection map), and to report the coordinates in Paxinos space. [$^{18}$F]FDG PET images were automatically co-registered with a [$^{18}$F]FDG rat brain template that was created in-house using the methodology described by Casteel et al 2006[20]. Extra-cerebral regions were removed from the PET images by application of a mask. The PET images were smoothed with a 1.2 mm isotropic Gaussian kernel. Global brain uptake differences between rats were normalized by proportional scaling relative to the mean whole brain [$^{18}$F]FDG uptake.

Voxel-based analysis was performed using two-sample t-tests (paired within-groups and unpaired between groups). *T*-map data were interrogated at $p<0.005$

uncorrected with an extent threshold of 200 voxels. Only clusters with a corrected family-wise error (FWE) $p<0.05$ were considered significant.

### Statistical analysis

All data are expressed as mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics 22 for Windows. Differences between rest and stress whole brain [$^{18}$F]FDG uptake were analyzed with a paired t-test. Between-group effects of treatment on the body weight, behavior in the FST and whole brain [$^{18}$F]FDG uptake were analyzed by one-way ANOVA followed by a Bonferroni post hoc test. Between-group statistical analysis of differences in resting state [$^{18}$F]FDG PET data on day 14 was not performed on OVX-FLX and OVX-ESC groups, because these treatments started at day 15. Effects were considered significant when the probability (p) was $<0.05$.

## Results

### Body weight and temperature

The increase in bodyweight of the rats at day 7 and 14 post-ovariectomy is presented in figure 2A. Rats in the OVX control, OVX-FLX and OVX-ESC groups showed a similar increase in the body weight over time, with an average increase of 18±7 g at day 7 and 43±9 g at day 14. Antidepressant treated rats did not show any significant differences in the change in the body weight at either time point, as compared to OVX control rats ($p>0.05$). In contrast, OVX-7d-E2 rats showed a comparable increase in bodyweight as OVX control rats on day 7 (17±6 g), but a significantly smaller increase in body weight on day 14 post-ovariectomy (6±5 g, $p<0.001$). OVX-14d-E2 rats showed a decrease in body weight, rather than an increase, at both time points post-ovariectomy (-15±5 g and -4±6 g on day 7 and 14 post-ovariectomy, respectively, $p<0.001$).

A representative measurement of the body temperature of an OVX control rat, from day 12 to day 16 post-ovariectomy, is displayed in figure 2B. For all rats a normal day and night rhythm was observed with an average body temperature of 37.76±0.17 $^{o}$C. During the experimental period a reduction in the body temperature was observed at three time points: i.e. during the resting state [$^{18}$F]FDG scan (34.18±1.04 $^{o}$C), during the pretest-FST (33.71±0.57 $^{o}$C) and during the test-FST directly followed by the stress [$^{18}$F]FDG scan (33.04±1.58 $^{o}$C). The reductions in body temperature were not significantly different between any of the groups ($p>0.05$).

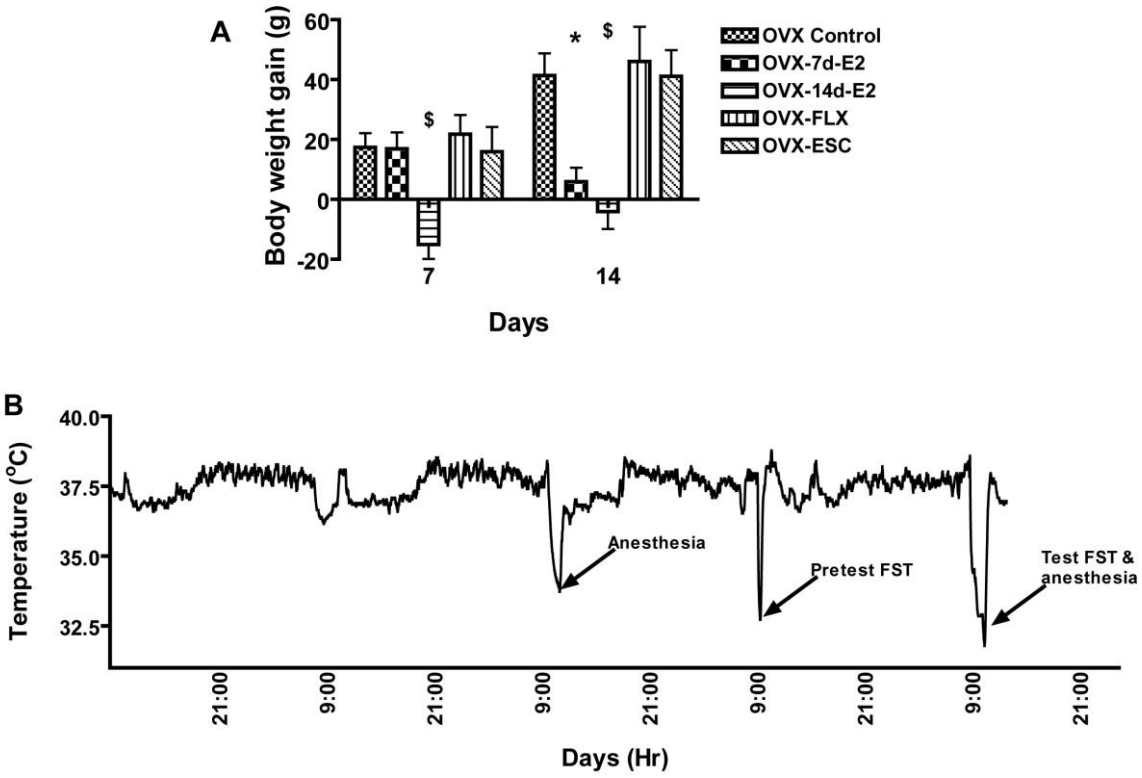


**Figure 2:** Body weight gain and body temperature. A) Influence of ovariectomy and different treatments on the body weight of the rats during the whole experimental period, and B) a representative record of body temperature measurements from day 12-16 of the experiment. Data are presented as mean±SD (n=8 per group). Significant differences compared to OVX control rats are indicated by *($p<0.001$) and $ ($p<0.001$).

## Circulating estradiol levels

The concentrations of circulating estradiol at day 16 post ovariectomy were 16±3, 661±499, and 339±275 pg/ml in OVX, OVX-7d-E2 and OVX-14d-E2 rats, respectively. The levels of estradiol in OVX-7d-E2 were significantly higher than those in OVX rats ($p<0.01$), but not significantly different from those in OVX-14d-E2 rats ($p=0.2$).

## Behavior

The results of the FST are presented in figure 3. In the first 5 min of the test-FST, OVX control rats spent 50% of time on floating, 30% of time on swimming and 20% of time on climbing. OVX-14d-E2 rats showed a significant increase in the time spent on climbing (76%; $p<0.05$) and a decrease in the time spent on floating (36%; $p<0.05$), when compared to OVX control rats. Rats in the OVX-7d-E2, OVX-FLX and OVX-ESC groups did not show any significant differences in the time spent on climbing, floating or swimming, when compared to OVX rats.

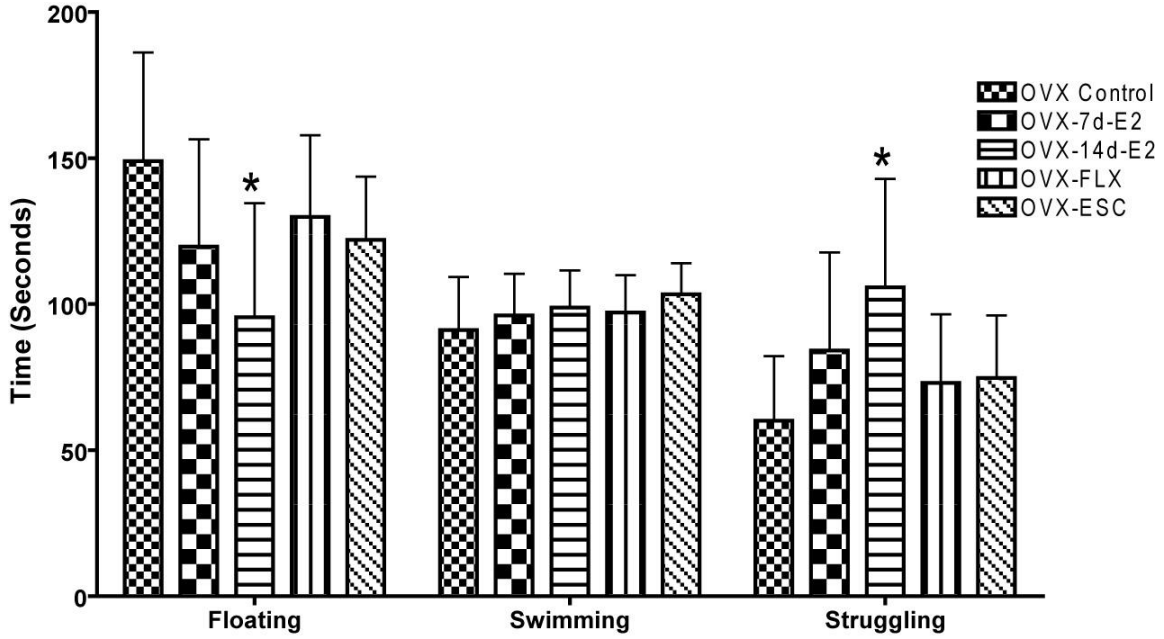


**Figure 3:** Effect of treatment on FST behavior: Time spent on a specific behavior during the first 5 min of the FST. Statistical analysis was performed by one-way ANOVA followed by a Bonferroni post hoc test. Data are presented as mean±SD (n=8). Significant differences are indicated by *($p<0.05$) compared to OVX control rats.

## Whole brain metabolism

Representative resting state and stress [$^{18}$F]FDG PET images are presented in figure 4A. In the resting state PET images, the effect of immediate and delayed estradiol replacement on global brain metabolism was investigated. Quantitative analysis of resting state [$^{18}$F]FDG PET (Figure 4B) revealed a significantly lower whole brain [$^{18}$F]FDG uptake in OVX-14d-E2 rats than in OVX control animals ($p<0.05$), but no significant effect in OVX-7d-E2 rats. Subsequent exposure of the rats to the FST caused a significant reduction in whole brain [$^{18}$F]FDG uptake in all groups, except for OVX-FLX rats (-43%, $p<0.001$ for OVX; -37%, $p<0.05$ for OVX-7d-E2; -37%, $p<0.001$ for OVX-14d-E2; -37%, $p<0.05$ for OVX-ESC; -25%, $p=0.24$ for OVX-FLX). No significant differences in the whole brain [$^{18}$F]FDG uptake between the treatment groups and the OVX control group were observed during stress ($p>0.05$). However, a significantly lower whole brain uptake of [$^{18}$F]FDG was observed during stress in OVX-14d-E2 rats than in OVX-FLX rats ($p<0.05$), which may reflect that the working mechanism of fluoxetine and estradiol on depressive-like behavior is different in this model.

## Regional brain metabolism

Results of voxel based analysis of [$^{18}$F]FDG uptake after global normalization are presented in figure 5. To investigate the effect of estradiol replacement, PET scans in the resting state were compared, but no statistically significant between-group differences in regional [$^{18}$F]FDG uptake were found between the OVX control group and the estradiol replacement groups (data not shown).

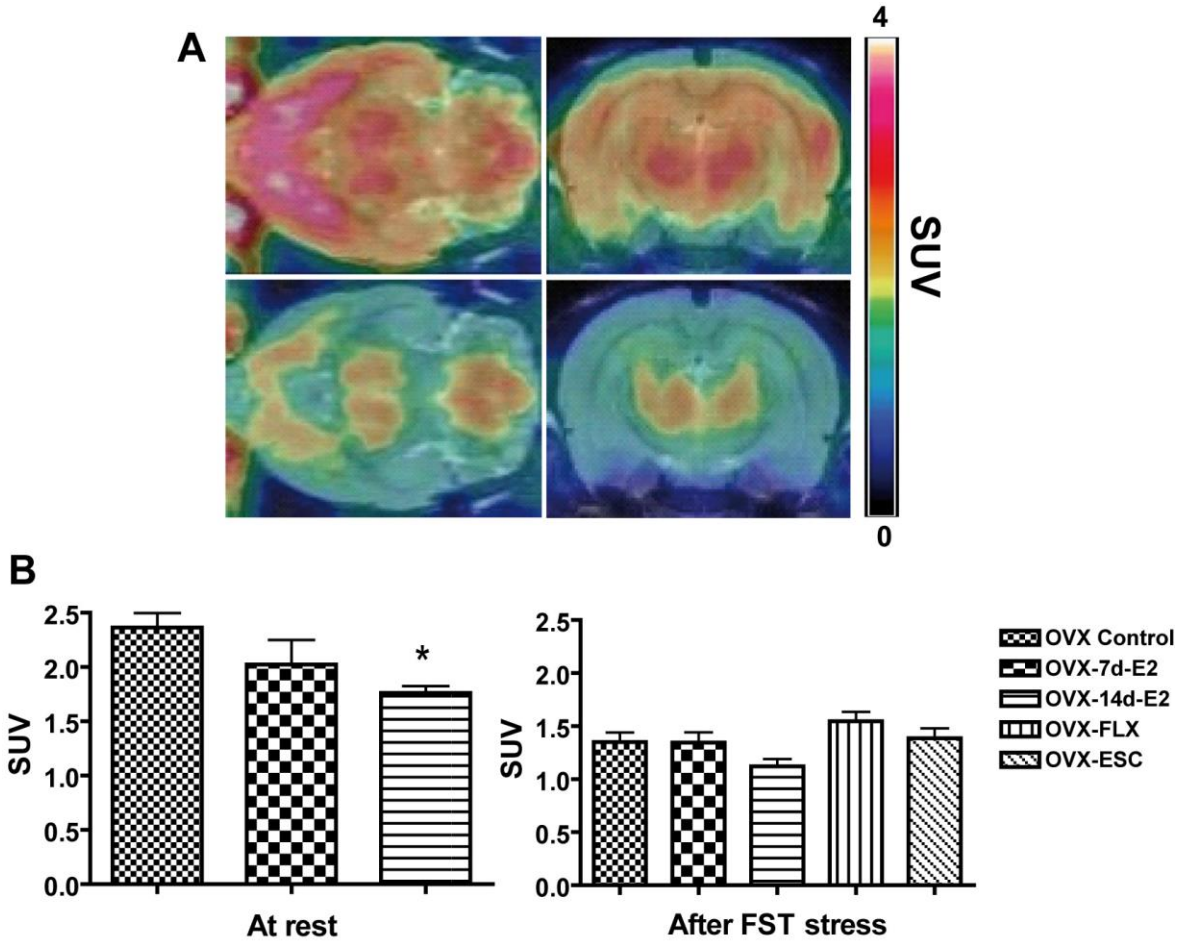


**Figure 4:** A) Representative axial and coronal [$^{18}$F]FDG PET images co-registered on MRI template of a rat at the resting state (top) and during stress (bottom). Images represent uptake between 30 min at 45 min after [$^{18}$F]FDG injection, and B) whole brain [$^{18}$F]FDG uptake in resting state and during stress, expressed as SUV. Data are presented as mean + SD. Significant differences compared to OVX rats are indicated by *($p<0.05$).

Comparison of resting state with stress PET scans for all groups combined revealed that exposure to the FST (stress) had a statistically significant main effect on brain metabolism in multiple brain regions (Figure 5A). Increased [$^{18}$F]FDG uptake during stress was found in the ventral tegmental area (VTA), substantia nigra, pons, cerebellum and medulla, while a decreased tracer uptake during stress was found in the striatum (caudate putamen and globus pallidus), amygdala, hippocampus, superior colliculus and most of the cortical regions, including the insular, frontal association, and somatosensory cortices. The [$^{18}$F]FDG PET results of the within-group comparison between resting state and stress for individual treatment groups are presented in Figures 5B to 5F and Table 1 (brain areas with increased uptake) and 2 (brain areas with decreased uptake). In OVX-FLX rats, no significant regional effect of FST-induced stress on [$^{18}$F]FDG uptake was observed at all (Figure 5E). In all other groups, cerebellum and medulla were found to be common regions with increased [$^{18}$F]FDG uptake, and striatum, amygdala, hippocampus, and superior

colliculus were common regions with decreased [$^{18}$F]FDG uptake during stress, as compared to the resting state.

Between-group comparison of the stress [$^{18}$F]FDG PET scans showed that OVX-14d-E2 rats had higher brain [$^{18}$F]FDG uptake in the periaqueductal grey, superior colliculus and cerebellum and lower [$^{18}$F]FDG uptake in the caudate putamen and corpus callosum than OVX control rats during stress (Figure 5G, and Table 3). None of the other treatment groups showed any statistically significant differences in regional [$^{18}$F]FDG uptake during stress, when compared to the OVX control group (data not shown).

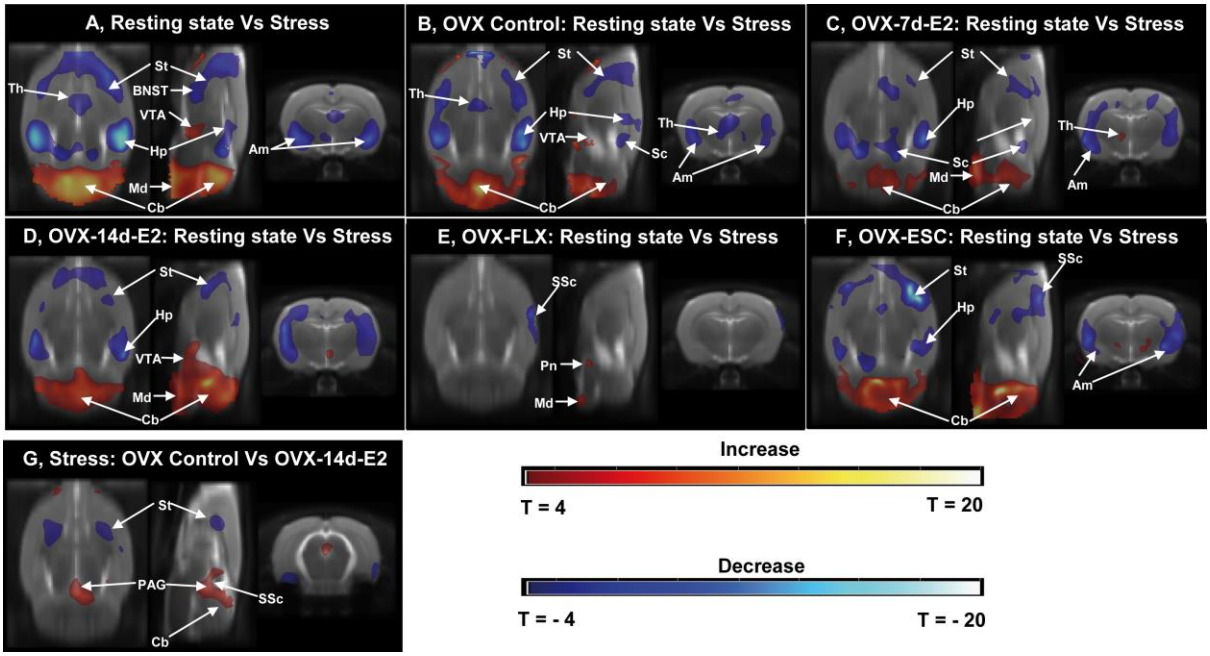


**Figure 5:** Voxel base analysis showing significant changes in brain [$^{18}$F]FDG uptake: (A-F) Resting state PET acquired at day 14 vs. stress PET scans acquired at day 16 for (A) all the groups together, (B) OVX rats, (C) OVX-7d-E2 rats, (D) OVX-14d-E2 rats, (E) OVX-FLX rats, and (F) OVX-ESC rats. (G) Stress PET of OVX-14d-E2 rats acquired at day 16 compared to OVX controls (Blue: deactivation; Red: activation; Am, Amygdala; BDNS, Bed nucleus of stria terminalis; Cb: Cerebellum; Hp, Hippocampus; Md, Medulla; PAG, Periaqueductal grey; Po, Pons; St: Striatum; SC, superior Colliculus; Th, thalamus; VTA, ventral tagmental area).

## Discussion

In this study, we demonstrated that estradiol treatment started immediately after ovariectomy prevented body weight increase, reduced depressive-like behavior and decreased global brain glucose metabolism. In contrast, delayed estradiol replacement initiated one week after ovariectomy could neither reduce depressive-like behavior, nor induce changes in brain glucose metabolism. However, delayed estrogen replacement did attenuate the gain in body weight induced by ovariectomy.

During the menopausal transition, women have an increased risk of abdominal fat accumulation, which is accompanied by an increase in body mass index. This effect was ascribed to the hormonal changes during menopause. There is substantial

evidence that estrogen replacement can prevent the increase in body weight after menopause[21]. In our study, ovariectomy caused an increase in body weight that exceeded the normal increase in body weight in this rat strain (8-12 g per week). The increase in body weight could be prevented by estrogen replacement, indicating that this animal model can mimic a physiological change of estrogen depletion and replacement in post-menopausal women.

Our findings on the effect of estradiol replacement on behavior are also consistent with clinical studies that have demonstrated that estradiol replacement could alleviate depressive signs in peri-menopausal women[22], provided that treatment was started in time. Evidence strongly suggests that initiation of estrogen replacement several years after menopause does not have any beneficial effect anymore[23]. Apparently, there is a narrow window of opportunity for effective estrogen replacement, suggesting that only timely started estrogen treatment can prevent depressive-like symptoms. In our study, estradiol replacement could not reverse depressive-like behavior after ovariectomy when treatment was started with a delay of only one week. However, an alternative explanation for the lack of efficacy of delayed estradiol replacement could be that the rats in our study were treated with estradiol for only 9 days, which may have been too short to reverse the depressive-like behavior induced by ovariectomy.

**Table 1:** Voxel based analysis results showing the brain regions with statistically significant increase in [$^{18}$F]FDG uptake during stress compared to resting state. T-maps were interrogated with a cluster threshold of 200 and corrected FWE of $p<0.05$ .

| Groups | Brain region | Peak value Coordinates | | | T-value |
|---|---|---|---|---|---|
| All rats | Medulla | 1.5 | -12 | -10 | 18.02 |
| | Cerebellum | -0.2 | -10.8 | -5 | 17.72 |
| | Substantia nigra | -2 | -5 | -7.5 | 8.08 |
| | Mesencephalic region | 1.7 | -6.2 | -7 | 8.67 |
| | Ventral Tegmental Area (R) | 1.5 | -5.4 | -7.4 | 7.78 |
| | Ventral Tegmental Area (L) | -1.8 | -5.6 | -7.4 | 7.66 |
| | Pons | 0.1 | -10.6 | -10.2 | 14.21 |
| OVX | Olfactory Tubercle (R) | 2.1 | 2.5 | -8.2 | 11.71 |
| | Cerebellum | -0.4 | -12.4 | -8.4 | 20.58 |
| | Medulla | -1.5 | -13.5 | -8 | 18.52 |
| | Entorhinal Cortex (L) | -5 | -8.4 | -5.8 | 18.35 |
| OVX-7d-E2 | Pons | -0.06 | -10.4 | -10 | 11.43 |
| | Cerebellum | -1.6 | -10.6 | -5.2 | 8.51 |
| OVX-14d-E2 | Cerebellum | -0.6 | -10.4 | -5.6 | 17.71 |
| | Medulla | 1.5 | -11.6 | -9.6 | 10.9 |
| OVX-FLX | - | - | - | - | NS |
| OVX-ESC | Medulla | 0.7 | -11 | -7.2 | 32.91 |
| | Cerebellum | 0.7 | -10.4 | -4.2 | 19.1 |

**Table 2:** Voxel based analysis results showing the brain regions with statistically significant decrease in [$^{18}$F]FDG uptake during stress compared to resting state. T-maps were interrogated with a cluster threshold of 200 and corrected FWE of $p<0.05$.

| Groups | Brain region | Peak value coordinates | | | T-value |
|---|---|---|---|---|---|
| All rats | Hippocampus, Ventral | 5.7 | -6.2 | -5.6 | -23.21 |
| | Hippocampus, posterodorsal | -5.8 | -5.4 | -5.2 | -20.59 |
| | Corpus Collosum | 5.7 | -5 | -3.8 | -16.45 |
| | Caudate Putamen (R) | 4.9 | -0.8 | -5.5 | -14.93 |
| | Superior colliculus (R) | 1.5 | -8.5 | -3 | -14.82 |
| | Amygdala (R) | -4.8 | -1.5 | -7.4 | -13.84 |
| | Amygdala (L) | -5.4 | -3.8 | -7.2 | -13.59 |
| | Superior Colliculus (L) | -1.5 | -8.4 | -3.2 | -11.78 |
| | Somatosensory Cortex (L) | -3.8 | 1.8 | -3.5 | -11.48 |
| | Frontal Association Cortex | -0.5 | 5 | -3 | -11.15 |
| | Insular Cortex (L) | -3 | 3 | -3.8 | -10.35 |
| | Visual Cortex (R) | 3.1 | -5.4 | -1.4 | -9.49 |
| | Globus pallidum (R) | 3.5 | -0.8 | -7 | -10.27 |
| | Globus pallidum (L) | -0.34 | -0.8 | -7.2 | -10.1 |
| OVX | Olfactory Nuclei (R) | 1.1 | 5 | -5.2 | -18.74 |
| | Hippocampus, Posterodorasal (L) | -5.6 | -5.2 | -4.4 | -15.1 |
| | Corpus collosum (L) | -5.8 | -5.8 | -4.8 | -14.74 |
| | Auditory cortex (R) | 5.5 | -4.5 | -4.5 | -13.52 |
| | Hippocampus, Posterodorasal (R) | 5.9 | -5.4 | -4.5 | -12.34 |
| | Hippocampus, Ventral (L) | -4.8 | -5.6 | -5.8 | -11.98 |
| | Caudate Putamen (R) | 5.1 | -1.2 | -6.4 | -11.81 |
| | Superior Colliculus (R) | 2.1 | -8.2 | -3 | -11.76 |
| | Olfactory Tubercle (L) | -1.2 | 4.6 | -5.4 | -10.94 |
| | Medial Frontal Cortex (R) | 1.1 | 4.6 | -2.6 | -10.52 |
| | Orbitofrontal Cortex | -0.4 | 5 | -3.2 | -10.23 |
| | Parietal Association Cortex (R) | 3.3 | -4.2 | -1.2 | -9.31 |
| OVX-7d-E2 | Hippocampus, Subiculum (L) | -5.6 | -7 | -5 | -13.64 |
| | Corpus Callosum (L) | -5.8 | -6.4 | -3.8 | -13.29 |
| | Amygdala (L) | -4.8 | -2.2 | -7.2 | -10.35 |
| | Ventral Pallidum (L) | -2.6 | -0.6 | -7.4 | -10.53 |
| | Cingulate Cortex | 0.1 | -0.4 | -3 | -7.51 |
| | Caudate Putamen (L) | -1.8 | -0.4 | -4.2 | -6.67 |
| OVX-14d-E2 | Temporal Association Cortex (L) | -5.8 | -7.4 | -3.5 | -14.25 |
| | Accumbens Shell (L) | -3.2 | -0.6 | -7.6 | -12.56 |
| | Caudate Putamen (L) | -4.4 | -0.2 | -6.8 | -12.11 |
| | Corpus Callosum (R) | 5.9 | -5 | -4 | -11.24 |
| | Caudate Putamen (R) | 4.9 | -1.2 | -6.8 | -11.15 |
| | Amygdala (R) | 4.9 | -3.8 | -7 | -10.51 |
| | Hippocampus, Ventral (R) | 5.7 | -5.6 | -5.8 | -10.48 |
| | Visual Cortex (R) | 3.9 | -6.2 | -1.6 | -10.34 |
| | Amygdala (L) | -5 | -3.5 | -8 | -9.55 |
| | Auditory Cortex (R) | 5.3 | -6.4 | -4 | -9.16 |
| | Superior Colliculus (R) | 1.1 | -7.2 | -3 | -9.79 |
| | Entrosplenial Cortex | 0.5 | -2 | -1.4 | -5.42 |
| OVX-FLX | - | | | | NS |

table continued..

**Table 2 (continued):** Voxel based analysis results showing the brain regions with statistically significant decrease in [$^{18}$F]FDG uptake during stress compared to resting state. T-maps were interrogated with a cluster threshold of 200 and corrected FWE of $p<0.05$.

| Groups | Brain region | Peak value coordinates | | | T-value |
|---|---|---|---|---|---|
| OVX-ESC | Hippocampus, Ventral (R) | 5.9 | -6.2 | -6.2 | -28.76 |
| | Caudate Putamen (L) | -4.8 | 0.4 | -4.6 | -27.37 |
| | Piriform Cortex (R) | 5.7 | -1.8 | -8 | -18.16 |
| | Auditory Cortex (L) | -6.6 | -3.8 | -5.2 | -15.27 |
| | Motor Cortex (L) | -2.2 | 1.8 | -2 | -15.2 |
| | Motor Cortex (R) | 3.1 | 2.6 | -2.8 | -13.39 |
| | Cingulate Cortex | -0.4 | 1.4 | -1.6 | -12.8 |
| | Hippocampus, Ventral (L) | -4.4 | -5.4 | -6.8 | -11.85 |
| | Somatosensory Cortex (R) | 4.5 | 2.2 | -4.4 | -11.05 |
| | Insular Cortex (R) | 5.1 | -0.2 | -7 | -10.29 |
| | Visual Cortex (R) | 5.5 | -6.4 | -2.8 | -10.1 |
| | Temporal Association Cortex (R) | 5.5 | -7.6 | -3.8 | -9.95 |
| | Superior Colliculus (R) | 1.1 | -9 | -3.2 | -12.17 |

**Table 3:** Voxel based analysis results showing the brain regions with statistical significant differences in [$^{18}$F]FDG uptake in the stress [$^{18}$F]FDG PET scan in OVX-14d-E2 rats compared to OVX controls.

| Increase/Decrease | Brain region | Peak value coordinates | | | T-value |
|---|---|---|---|---|---|
| Increase | Superior Colliculus | -0.2 | -5 | -3.8 | 4.55 |
| | Cerebellum | -2.8 | -9.5 | -5.6 | 3.22 |
| | Periaqueductal grey | 0.6 | 7.8 | -5.1 | 4.72 |
| Decrease | Caudate Putamen (R) | 2.7 | -0.2 | -4.2 | -4.51 |
| | Corpus Callosum | -0.2 | 0.6 | -2.4 | -3.38 |

[$^{18}$F]FDG PET was used to evaluate the effect of estradiol replacement and stress on brain glucose metabolism of ovariectomized rats. The effect of the FST was evaluated by comparing resting state and stress PET scans. When all treatment groups were pooled together, a large global decrease in [$^{18}$F]FDG uptake was found after the stress challenge in the FST, as compared to resting state. This effect was possibly related to reduced global delivery of [$^{18}$F]FDG to the brain due to increased muscular activity and temperature maintenance during swimming. When the PET scans were corrected for the global differences in whole brain [$^{18}$F]FDG uptake, a significant increase in focal brain metabolism in the pons, cerebellum and medulla was apparent during stress when compared to the resting state. These brain regions are also associated with general processes of emotion and also coordination of muscular movement, maintenance of balance and posture in any (stressful) condition. Therefore, these differences can be partly ascribed to both the emotional response and the physical activity of the animals during the FST. In addition, several brain areas exhibited decreased relative brain glucose metabolism in the stress PET scans relative to resting state, in particular in hippocampus, amygdala, superior colliculus, striatum, and most of the cortical regions including the insula. An increased glucose

metabolism during stress is found in the VTA, which is located in the ventral part of the midbrain, and is the nucleus from which exclusively the inhibitory neurotransmitter dopamine is released. The VTA projects to striatum, amygdala, and (frontal) cortex by mesostriatal, mesolimbic and mesocortical projections, respectively. The observed stress-induced relative hyper-activation of the glucose uptake in VTA and the decreased uptake in striatum, amygdala, and (frontal) cortex might be explained by these interconnections. Our findings are only partly consistent with the findings of Jang et al.[24] who did a similar study on brain metabolism during the FST, using [$^{18}$F]FDG PET. They observed a higher metabolism in the striatum and cerebellum and a lower metabolism in the hippocampus, inferior colliculus and insula of healthy control rats during the FST, when compared to resting state. While Jang et al.[24] found a higher metabolism in the striatum, we observed that the metabolism in the striatum was decreased. These differences may be due to the gender difference, as Jang et al. studied male rats, whereas females were used in our study.

The effect of estrogen replacement after ovariectomy on brain glucose metabolism was investigated both in resting state and during stress, because stress could potentially aggravate the effects of estrogen depletion and, as discussed above, the FST by itself already has a significant effect on brain glucose metabolism. Between-group comparison of resting state global brain metabolism revealed that immediate, but not 1-week delayed, estradiol replacement caused a significant reduction in whole brain [$^{18}$F]FDG uptake. After global normalization of tracer uptake, no regional differences in focal [$^{18}$F]FDG uptake could be observed. These finding are not in line with a recent animal study that found an increase, rather than a decrease, in glucose metabolism 3 weeks after ovariectomy. This increase in glucose metabolism could be reversed by immediate estradiol replacement, but not by 3-weeks delayed estradiol replacement[25]. Our results on resting state glucose metabolism also seem to be in conflict with clinical studies that have demonstrated a reduced regional cerebral blood flow and glucose metabolism in patients with major depression[10]. If estrogen deprivation is associated with depression, one would expect that immediate estrogen replacement after ovariectomy would result in an increase glucose metabolism, rather than the decrease that we observed.

In contrast to the resting state PET, no significant differences in global [$^{18}$F]FDG uptake between the estradiol replacement groups and untreated ovariectomized rats were found in the PET scans acquired during stress. After global normalization of whole brain [$^{18}$F]FDG uptake, however, regional differences in relative brain metabolism were observed between untreated ovariectomized rats and ovariectomized rats that immediately received estradiol replacement. Immediate estradiol replacement led to an increase in relative glucose metabolism in the periaqueductal grey, superior colliculus and cerebellum and a decrease in relative glucose metabolism in caudate putamen and corpus callosum. The periaqueductal grey is an area of the brain that is known to initiate the processes of escapable (active) and inescapable (passive) coping behavior when exposed to a particular stressor. Together with the medial hypothalamus, superior and inferior colliculi, the

periaqueductal grey forms the so-called brain defense system or fight and flight system[26]. Immediate estradiol replacement increased the duration of climbing in the FST, which is in agreement with the flight/fight behavior that would result from the activation of the periaqueductal grey, probably in the dorsal part[27].

In this study, ovariectomized rats exposed to sub-acute treatment of the SSRIs fluoxetine and escitalopram were included as positive control groups for behavioral effects. A remarkable finding in our study was that sub-acute treatment with these SSRIs could not produce any significant effect on depressive-like behavior. In contrast to our results, other studies have demonstrated that sub-chronic treatment with SSRIs can have an effect on depressive-like behavior as measured in the FST[12,28]. This apparent discrepancy could be due to the duration of treatment in our study, which may have been insufficient to induce the desensitization of the serotonergic receptors or transporters[29]. Another explanation for the lack of efficacy of the SSRIs could be gender differences between the studies. In contrast to our study, the effect of sub-chronic SSRIs treatment is usually investigated in male rats. Male and female rats respond differently to stress and to drug treatment, both behaviorally and neurochemically[30]. An alternative explanation could be that anti-depressant activity of the SSRIs in females could be mediated by circulating estrogens[31–33]. Since our study was performed on ovariectomized rats, circulating estrogen levels were low. Several studies in rodents and humans have shown that antidepressants may show little effect in the absence of circulating estrogens[34–36].

Sub-acute treatment with fluoxetine and escitalopram had different effects on brain glucose metabolism. While the escitalopram-treated group showed similar regional differences in relative brain metabolism between resting state and stress PET as the ovariectomized controls and the estrogen replacement groups, no significant effect of FST on regional brain metabolism was observed in fluoxetine-treated rats. Our results differ from those found by Jang and coworkers[12], who found that the FST caused a relative increase in brain metabolism in cerebellum, motor and sensory cortex, and a decrease in amygdala and piriform cortex of fluoxetine treated rats during the FST, as compared to resting state. This discrepancy could be due to gender differences between both studies. The differences in FST-induced changes in brain metabolism between escitalopram and fluoxetine observed in our study may be due to differences in the mechanism of action between the SSRIs. Escitalopram is a very selective SSRI, whereas fluoxetine also interferes with norepinephrine and dopamine reuptake[37] and can bind to serotonin 2C[38] and sigma1 receptors[39]. In addition, escitalopram has superior efficacy and needs less time to produce its antidepressant effect[40]. Based on these pharmacological differences, it can be speculated that fluoxetine treatment affected different neurotransmitter systems, causing different effects on brain glucose metabolism.

## Conclusion

Our study showed that immediate estradiol replacement after ovariectomy prevented the development of depressive-like behavior in rats and affected regional brain glucose metabolism during stress. The regional pattern of affected brain glucose

metabolism suggests that immediate estradiol replacement influences motivational cues by stimulating monoaminergic (inhibitory) projections of the midbrain to the basal ganglia and cortex. In contrast, delayed estradiol replacement, initiated one week after ovariectomy, did not have any significant effect on depressive-like behavior or brain glucose metabolism during stress. In the present study, 24 h treatment with standard antidepressant drugs did not affect depressive-like behavior. These results suggest mechanistically that estrogens may be a preferable biological therapy for post-menopausal depression as compared to SSRIs, but that the appropriate timing of hormonal therapy will be very critical for success.

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